

## Efficiency and response of corn cultivars to nitrogen, associated or not with *Azospirillum brasilense*

Vanderlan Carneiro Dias<sup>1</sup>, Joênes Mucci Peluzio<sup>1</sup>, Flávio Sérgio Afférri<sup>1</sup>, Maria Dilma de Lima<sup>1</sup>, Domingos Bonfim Ribeiro dos Santos<sup>1</sup>, Guilherme Benko<sup>1</sup>, Weder Ferreira dos Santos<sup>2\*</sup>, Layanni Ferreira Sodrê Santos<sup>2</sup>, Adriano Silveira Barbosa<sup>2</sup>, Lucas Alves de Faria<sup>2</sup>

<sup>1</sup>Federal University of Tocantins, Palmas, Tocantins, Brazil.

<sup>2</sup>Federal University of Tocantins, Gurupi, Tocantins, Brazil.

\*Corresponding Author

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nitrogen.

**Abstract**— Green corn is an economic and social alternative for small and medium-sized farmers. However, the high cost of the inputs, especially in relation to the use of nitrogen, has advanced studies with nitrogen-fixing bacteria. This study evaluated commercial maize cultivars for the production of green ears, responsive and efficient to nitrogen use, with and without application in *Azospirillum brasilense* seeds. Two trials were installed in the agricultural years 2019/20 and 2020/21 in sowings carried out on 12/04/2019 and 10/12/2020 in this order, at the Federal University of Tocantins, Palmas-TO. In each trial, the experimental design was randomized blocks, with three replications, being the treatments arranged in subdivided plots, where the treatments involving the processes with inoculation of seeds with the bacterium *Azospirillum* (C Az) and without inoculation of seeds (S Az) were allocated in the plots, two doses of nitrogen (30 and 120 kg ha<sup>-1</sup> N, considered as low and high N, respectively) and in the subplots eight corn cultivars were allocated in the subplots. Joint variance analysis was performed for each of the seed inoculation processes (C Az and S Az) and then the efficiency and response of corn cultivars were studied for the use of N for each process. The inoculation of seeds with the bacterium promoted changes in the efficiency and response of cultivars regarding the use of nitrogen. The cultivar BRS-3046 was the most important for the cultivation of green ears, since it was efficient and responsive to the use of nitrogen with *Azospirillum brasilense*.

### I. INTRODUCTION

The production of green corn (*Zea mays*) is a source of income and food, both for human and animal feed, being used by the consumer market in grain or in natura "green ears" and in the canned industry [1]. In Brazilian cuisine, it is marketed in free fairs, grocery stores and supermarket

chains and even on the borders of highways in the forms of roasting, cooked etc. [2].

In the Northern region of Brazil, specifically in the state of Tocantins, the low productivity of corn occurs, among other factors, due to the presence of high temperatures, low technological level, scarcity of

improved seeds, and the conditions of abiotic stresses, such as climatic and nutritional variations, in the latter case, mainly related to N [3].

The use of nitrogen fertilizers is one of the factors responsible for high corn productivity. According to Lemaire & Gastal [4], N is the element required in greater quantity by corn and the most limiting for grain yield. However, its continuous use out of production costs related to its acquisition, transportation, application installment, etc. [5], [6].

Obtaining genotypes with greater efficiency in the use of nitrogen (EUN) would be a desirable alternative for capitalized agriculture and low use of insums, aiming to reduce waste and scarcity of this mineral element, which could generate economic, environmental, public health and food safety problems [7]. An alternative for the supply of N to plants would be to use plant growth-promoting bacteria (BPCP) for biological nitrogen fixation (FBN), via *Azospirillum brasilense*, which is considered a cheap, clean and sustainable alternative in the supply of N, with benefits for current agriculture [8], besides contributing to the reduction of greenhouse gas emissions [9].

However, there are still contradictions regarding the efficiency of the use of Inoculation of *Azospirillum brasilense* in corn seeds. While [10], it highlights that efficiency can increase by up to 30% in productivity and promote a reduction of up to 15% of nitrogen fertilization [9], reports not obtaining increases in plants when inoculated with bacteria and nitrogen doses. According to Quadros et al. [11], the success of inoculation may vary due to some factors, such as soil type, soil climate and plant genotype.

Several researchers have sought to obtain genotypes with higher EUN for corn ([12], [13], [14], [15], [16], [17]. However, there are few studies involving corn genotypes, responsive and efficient to nitrogen use, after inoculation of seeds with *Azospirillum brasilense*, as well as comparisons involving EUN in cultivars with seeds inoculated and not inoculated with *Azospirillum*.

Thus, the present work was carried out with the objective of studying commercial cultivars of corn for the production of green ears, efficient and responsive to the use of nitrogen, with and without application in the seeds of the bacterium *Azospirillum brasilense*.

## II. MATERIAL AND METHODS

Two trials were installed in the experimental area of the Federal University of Tocantins in Palmas-TO (latitude 10°12'46"S, longitude 48°21'37"W and altitude of 260 m), one in the agricultural year 2019/20, in sowing held on

12/04/2019, and another in the agricultural year 2020/21, in sowing held on 10/12/2020.

The climate is tropical humid (Köppen, Aw) which contributes to the high temperatures in the region. During the periods of conduction of the tests, the average maximum, minimum and relative humidity were in the ranges of 35.9 °C; 26.33 °C and 65.96% RH respectively. The cumulative total rainfall in the period was 1,418 mm, with monthly averages of 354.5 mm [18], [19], [20].

The soil of the experimental area, according to the Brazilian Soil Classification System is considered as dystrophic Yellow Red Latosol [21]. Soil samples collected at a depth of 0 to 20 cm, and their chemical analyses revealed the following: pH(CaCl<sub>2</sub>) 6.0; clay 15.5%; Site 5.9%; sand 78.6%; M.O 11.63 g dm<sup>-3</sup>; P (Mehlich-1) 9.92 mg dm<sup>-3</sup>; K 0.2 cmol dm<sup>-3</sup>; Ca 1.90 cmol dm<sup>-3</sup>; Mg 1.12 cmol dm<sup>-3</sup>; S.B 3.22 cmol dm<sup>-3</sup>; CTC 5.02 cmol dm<sup>-3</sup>, e V 64.14%.

The experimental design used in each assay was randomized blocks, with three replications. The treatments were arranged in subdivided plots, where the treatments were allocated in the plots with inoculation of seeds with *Azospirillum* (C Az) and without inoculation of seeds (S Az), in the subplots two nitrogen doses (30 and 120 kg ha<sup>-1</sup> N, considered as low and high N, respectively) and in the subsub plots eight corn cultivars, three simple hybrids (M-274, PR-27D28, AG 8088-PRO2), two double hybrids (BRS-2022, AG-1051), two triple hybrids (BRS-3046, BM-3061) and a variety of open pollination (Anhembi), all cultivars were acquired in the local trade.

The experimental plots consisted of four rows, with 3.0 m in length, spaced by 1.0 m totaling an area of 12,0 m<sup>2</sup>. At harvest, the two central rows were considered useful, discarding 0.50 m from the extremities.

The tillage was in conventional cultivation, without the need for catheches. At sowing, fertilization was performed in the groove with 70 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, and 48 kg ha<sup>-1</sup> of K<sub>2</sub>O, of potassium chloride.

Sowing was performed no-head in the groove, and the seeds were inoculated 30 minutes before planting with the bacterium *Azospirillum brasilense* (AbV5 and AbV6), being 100ml for every 25 kg of seeds, as recommended by the manufacturer. Population density was 50,000 plants per hectare [22].

Weed control was performed using post-emergent herbicide. Subsequently, weeding was performed. It was not necessary to control pests and diseases.

The cover fertilization was performed with ammonia sulfate (21% N), at doses of 30 (low N) and 120 kg ha<sup>-1</sup>

(high) in the between plots, being half applied in stage V4 and V8 (four and eight true leaves) [23].

In the useful area of each plot, the green ears were harvested as the grains presented 70% to 80% humidity, at stages R3 and R4 [24]. Then the ears were scattered, and the weight of each parcel converted into kg ha<sup>-1</sup>.

The data of each experiment were submitted to individual variance analysis and, when the homogeneity of variances was verified, the joint analysis was carried out for each of the seed inoculation processes (with and without *Azospirillum brasilense*). Next, the efficiency and response of maize cultivars regarding the use of nitrogen (N) was studied for each process, according to the methodology proposed by [25].

By this methodology, the efficiency corresponded to the yield measurement of green ears in each cultivar in the environment with low nitrogen (BN) (30 kg ha<sup>-1</sup> of N). On the other hand, the response to the application of N for each cultivar is represented by the formula below:

$$\text{Response (\%)} = (\text{RAN} - \text{RBN}) / (\text{DEN})$$

RAN = high yield N;

RBN = yield low N and;

DEN = difference between the doses applied (high N – low N, in kg ha<sup>-1</sup>).

The graphic representation was used in the Cartesian plane to classify the cultivars, and in the axis of the abscissas, the efficiency of the use of N is represented and

in the axis of the ordered the responses to its application. The point of origin of the axes corresponds to the average efficiency and average response of the cultivars. In the first quadrant are represented the efficient and responsive cultivars (I); in the second the Inefficient and Responsive (II); in the third the Non-Efficient and Non-Responsive (III) and in the fourth quadrant the Efficient and Non-Responsive (IV).

The medium of cultivars and nitrogen doses, in the processes with seed inoculation (C Az) and without seed inoculation (S Az) with the bacterium *Azospirillum brasilense*, were compared by Scott-Knott [26] test, at 5% significance using the statistical program SISVAR [27]. For the preparation of the graphics, the Origin Pro 8 program was used.

### III. RESULTS AND DISCUSSION

The medium average yield of green ears spread (kg ha<sup>-1</sup>) of the eight maize cultivars, in two nitrogen levels (30 and 120 kg ha<sup>-1</sup> N) and in two inoculation processes with *Azospirillum* (C Az) and without *Azospirillum* (S Az), as well as the efficiency and response indices are presented in Table 1.

Table 1: Average yield of green ears spread (kg ha<sup>-1</sup>) of eight green corn cultivars, in two levels of nitrogen (30 and 120 kg ha<sup>-1</sup> N) and two inoculation processes C Az (with *Azospirillum*) and S Az (without *Azospirillum*), in Palmas - TO, in the 2019/2020 and 2020/2021 harvests.

Cultivars	Low N (30 kg ha <sup>-1</sup> )		High N (120 kg ha <sup>-1</sup> )		Response	
	C Az	S Az	C Az	S Az	C Az	S Az
BRS-3046	9.037bA	8.920bA	10.784aA	9.974aA	19.41	11.71
Anhembi	7.719bB	8.200bB	9.343aB	9.085aB	14.04	9.83
M-274	7.811bB	8.678bA	9.606aB	9.261aB	19.94	6.47
PR-27D28	8.350bB	7.568bB	9.112aB	9.283aB	8.47	19.05
BRS-2022	8.670bA	8.184bB	9.540aB	9.323aB	9.67	12.65
BM-3061	9.474bA	8.300bB	10.214aA	9.974aA	8.22	18.60
AG-1051	9.136bA	9.380bA	9.614aB	10.071aA	5.31	7.68
AG 8088-PRO2	8.481bB	8.397bB	9.714aB	10.081aA	13.70	18.71
Medium	8.585b	8.578b	9.741 <sup>a</sup>	9.632a	12.35	13.09
General Medium	C Az 9.163 e S Az 8.977					
CV (%)	C Az 2.20 e S Az 2.29					

1- Medium between n doses, for the same inoculation process and for the same cultivar, followed by the same lowercase letter, in the line belong to the same statistical group by test [26], at 5% significance.

2- Medium between cultivars, for the same n dose and for the same inoculation process, followed by the same capital letter in the column belong to the same statistical group by test [26], at 5% significance.

Os coeficientes de variação CV (%) foram de C Az = 2.20 e S Az = 2.29 (tabela 1), o que indica uma ótima precisão na condução do ensaio experimental [28].

The comparative study between n doses in each inoculation process revealed that both in the C Az process and in the S Az process, all cultivars presented a higher yield of ears under high N ( $120 \text{ kg ha}^{-1}$ ).

The N is the nutrient with the highest demand in corn crop [29], being present in relevant functions in plant metabolism, such as protein synthesis, ionic absorption, photosynthesis, respiration, cell multiplication and differentiation, ultimately reflecting on plant characteristics related to growth and development, which directly or indirectly affect crop yield [30], [31]. [32] approximately 70 to 77% of what is absorbed is exported to the grains.

The results are in agreement with [17], who studied the efficiency and response of corn cultivars to nitrogen use in the south of the state of Pará, for silage production, and also verified a better performance of all cultivars when under cultivation in high N.

When comparing the cultivars, within each inoculation process and within each n dose, it can be observed that two groups of means were always formed, which differed in their composition. In this sense, when the seeds (C Az), both in high N and low N, the cultivars BRS-3046 and BM-3061 were the ones that stood out the most. On the other hand, in the process without inoculation (S Az), in high and low N, BRS-3046 and AG-1051 were the most productive.

It is emphasized the higher productivity of BRS-3046 and the lowest yields of the variety Anhembi and PR-27D28 in all doses of N and in all inoculation processes. The lowest yield of the cultivar Anhembi occurred because the varieties are known as open pollination cultivars and are obtained by the free pollination of a group of selected individuals. Thus, they are highly heterozygous and heterogeneous, presenting greater genetic variability and lower uniformity and productivity [33].

According to Fancelli [34], the agronomic and productive performance of corn cultivars are directly characterized by the different phenological phases of the crop, combined with its association with nutrients.

The efficiency and response to nitrogen fertilization of cultivars in the process without inoculation of seeds with *Azospirillum* (S Az), related to the production of green ears, are shown in Fig. 1a.

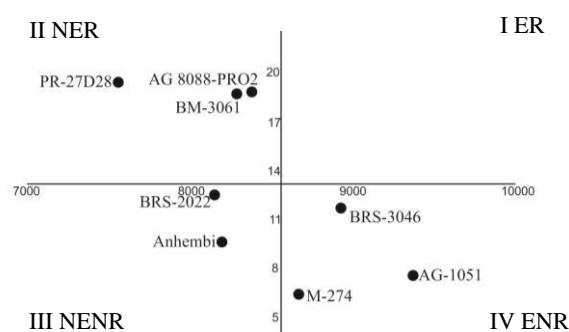


Fig. 1a S Azosp

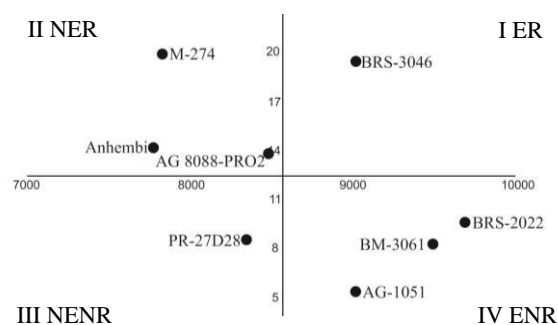


Fig. 1b C Azosp

Fig 1: Efficiency and response of nitrogen application associated with processes with and without *Azospirillum brasilense* (C Az and S Az) in eight green corn cultivars in Palmas - TO, in the 2019/2020 and 2020/2021 harvests.

The cultivars M-274, AG-1051 and BRS-3046 (Quadrant IV) were considered as efficient to the application of N, because they presented production of green ears under low N (BN) higher than the general average of cultivars in BN, and not responsive, that is, they are recommended for properties that adopt low technological level [35].

The cultivars AG 8088-PRO2, PR-27D28 and BM-3061 (Quadrant II) and Anhembi and BRS-2022 (Quadrant III) were classified as inefficient. Of these, the first three are responsive and the last two are non-responsive. Inefficient and responsive cultivars are indicated for use by producers with a high technological level [17]. On the



other hand, inefficient and non-responsive cultivars are not indicated for use in any agricultural properties [36].

In studies carried out in the South of the State of Pará that evaluated the efficiency and response of eleven commercial maize cultivars to nitrogen fertilization for silage production, they also obtained similar classifications for cultivars BRS-3046 and Anhembi, regarding nitrogen efficiency [17].

It is emphasized that without inoculation of seeds with *Azospirillum* (S Az), efficient and responsive cultivars (Quadrant I) were not obtained. This fact did not occur when the seeds were inoculated, revealing the importance of *Azospirillum brasilense* in the performance of the cultivars.

Corn germplasm consists of Creole breeds, adapted populations and introduced materials and is characterized by a wide genetic variability, which can interfere with the efficiency of the use of N [37].

Fernandes et al. [38], working with six maize cultivars, also showed significant differences in the efficiency of N utilization by plants, due to genetic variations among genotypes [39].

The efficiency and response to nitrogen fertilization of cultivars in the process with inoculation of seeds with *Azospirillum* (C Az), for the production of green ears, are shown in Fig. 1b.

The cultivars BRS-3046 (Quadrant I) and BRS-2022, BM-3061 and AG-1051 (Quadrant IV) were considered as efficient to the application of N. Of These, BRS-3046, highlighted for being efficient and responsive to the application of N and the others not responsive. According to Fidelis et al. [36] genotypes considered efficient and responsive are recommended for low to high technological agricultural crops, thus being economically viable for small farms and family farming.

The cultivars M-274, Anhembi and AG 8088-PRO2 (Quadrant II) and PR-27D28 (Quadrant III) were classified as inefficient. Of these, the first three are responsive and PR-27D28 non-responsive.

The comparative study between the processes of efficiency and n response with the inoculation of seeds (C Az) (Fig. 1b) and without inoculation of seeds (S Az) (Fig. 1a) revealed changes in the classifications of all cultivars, except for cultivars AG 8088-PRO2 and AG-1051.

The cultivars BM-3061 and BRS-2022, which were classified as inefficient to the application of N in the process without inoculation of seeds (S Az), in the process with inoculation of seeds with *Azospirillum* (C Az) were classified as efficient. This fact may have come from a greater availability of N via symbiotic fixation and, also,

from a greater absorption of nitrate available in the soil by the greater development of the root system of plants.

Bacteria of the genus *Azospirillum* spp., are able to stimulate plant growth by biological fixation of N and the increase in nitrate reductase activity [8], [40], as well as can alter the morphology and growth of the roots, enabling the exploration of a larger volume of soil [41], [42]. This higher root growth, which occurs due to the greater presence of indolic acid [43], may increase the absorption of minerals from the soil [44], including nitrate, P and K [41], [45] resulting in a higher production of dry matter, which combined with a greater accumulation of N in the plant, will promote increases in production [46], [47], [48].

For Chotte et al. [49], in soils with a deficiency of N, the biological fixation of N (FBN) can supply the deficiency of this nutrient in the soil, so that the occurrence of diazotrophic microorganisms in high numbers may be essential for the FBN is effective.

The efficiency of the use of *Azospirillum* spp., in the development of corn crop, has been the subject of research for several years. In this sense, [50], analyzing data from 22 years of field studies, they concluded that bacteria of the genus *Azospirillum* spp., tend to promote yield gains for corn crop in the most varied climate and soil conditions. These same authors also point out that the influence of *Azospirillum* spp., is not only related to the fact that these microorganisms act in biological nitrogen fixation, but also act as growth promoters, helping to increase the contact surface of the root system of plants, which culminates in greater absorption of water and nutrients by the roots.

Segundo Hungria [8], *Azospirillum* probably results in larger seedlings, with rapid initial growth, and in plants with a larger number of roots and longer roots, which provides a greater amount of dry matter in the shoot (28%) and a higher grain yield 7.1% (average of 221 Local). Also according to this same author, the bacteria of the genus *Azospirillum* are considered associative and excrete only a part of the nitrogen fixed directly to the associated plant, which will partially supply the needs of plants with N, which is still dependent on the hybrid used, edaphoclimatic conditions and adequate crop management. Thus, in addition to the fixation of nitrogen from associative bacteria, nitrogen fertilization is needed for the plant to obtain all the nitrogen necessary for its development.

Regarding the N response, the cultivars Anhembi, M-274 and BRS-3046 that were classified as non-responsive to the application of N in the process without inoculation of seeds (S Az), when seeds were inoculated with *Azospirillum* (C Az), were classified as responsive. On the

other hand, PR-27D28 and BM-3061, which were responsive to the application of N in the process without inoculation of seeds (S Az), became non-responsive when the seeds were inoculated with *Azospirillum* (C Az).

The genotype of the plant can influence the efficiency of N fixation Sala et al. [51], indicating a differential response of genotypes according to the form of inoculation used [52]. Thus, measures such as identification, selection and use of less demanding maize genotypes for element N are relevant tools from the economic and environmental point of view [53].

Salomone & Dobereiner [54] evaluating different maize genotypes inoculated with *Azospirillum* obtained different responses regarding inoculation under yield in production, highlighting that there are variations in the interactions between corn genotypes and diazotrophic bacteria.

Chotte et al. [49] when evaluating 32 maize cultivars for efficiency in nitrogen absorption and association with diazotrophic bacteria, they found that the occurrence of a high population of diazotrophic bacteria and the low response to nitrogen fertilization together with a large accumulation of N under conditions of low fertility, may indicate a promising cultivar for future studies of selection of efficient cultivars for cultivation in soils with low nitrogen availability.

Reis et al. [55] reported that, in many cases, the absence of response to inoculation of diazotrophic bacteria in grasses has been attributed to the use of inadequate strains. However, there is consensus that the genotype of the plant is the key factor to obtain the benefits derived from the BNF, combined with the selection of efficient strains.

#### IV. CONCLUSION

The inoculation of seeds with the bacterium *Azospirillum brasilense* promoted changes in the efficiency and response of cultivars regarding the use of nitrogen.

The cultivars efficient to the use of N, in the presence of *Azospirillum brasilense*, were BM-3061, BRS-2022 and AG-1051.

The cultivar BRS-3046 stood out and obtained its best increment for the cultivation of green ears, since it was efficient and responsive to the use of nitrogen in the presence of *Azospirillum brasilense*.

Breeding charts will be useful for the development of more efficient and nitrogen-responsive cultivars with the use of *Azospirillum*.

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